FORM PTO-1390 (REV 11-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 32409

U S APPLICATION NO (If known, see 37 CFR 1 5): 09/508828

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/05924	17 September 1998	20 September 1997

TITLE OF INVENTION
SYNTHETIC POLYPEPTIDE FOR DIAGNOSING AND TREATING PRION-RELATED DISEASES

APPLICANT(S) FOR DO/EO/US
Markus Moser, Bruno Oesch, and Carsten Korth

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U S C 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U S C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98. and Form PTO-1449.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

Computer Readable Sequence Listing and Statement Under 37 CFR 1.821(f) with computer disk and printed copy, small entity statement and 2 extra copies thereof.

"Express Mail" mailing label number EL142588625US

Date of Deposit March 16, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Linda Ibbett
Printed Name of Person Mailing Paper or Fee

Linda Ibbett
Signature of Person Mailing Paper or Fee

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/508427	PCT/EP98/05924	32409

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	CALCULATIONS	PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $760.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 0.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	18 - 20 =	0	X $18.00	$ 0.00	
Independent claims	9 - 3 =	6	X $78.00	$ 468.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,308.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 654.00	
SUBTOTAL =				$ 654.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 654.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 694.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 694.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0820 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

John P. Murtaugh
Pearne, Gordon, McCoy & Granger LLP
526 Superior Avenue East, Suite 1200
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John P. Murtaugh
SIGNATURE

John P. Murtaugh
NAME

34226
REGISTRATION NUMBER

09/508828
514 Rec'd PCT/PTO 16 MAR 2000

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Markus Moser, Bruno Oesch and Carsten Korth
International Application No.:	PCT/EP98/05924
International Filing Date:	September 17, 1998
Title:	SYNTHETIC POLYPEPTIDE FOR DIAGNOSING AND TREATING PRION-RELATED DISEASES
Docket No.:	32409

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the application prior to its examination as follows.

In the Specification:

Page 7 line 2, after "R_3 = Arg or Lys," insert --R_4 = Met, Val or Ala,--.

In the Drawings:

Please cancel original Figs. 1-4 and substitute therefore the enclosed Figs. 1-3 (provided on Sheets 1/2 and 2/2). We note that these Figs. 1-3 on Sheets 1/2 and 2/2 were attached as annexes to the International Preliminary Examination Report.

In the Claims:

On page 15, before claim 1, delete "CLAIMS" and insert therefore --WHAT IS CLAIMED IS:--.

In claim 4 line 1, delete "one of claims 1 through 3," and insert therefore --claim 1,--.

In claim 5 line 1, delete "one of claims 1 through 4," and insert therefore --claim 1,--.

In claim 6 line 1, delete "4 and 5,".

In claim 6 line 8, after "R_3 = Arg or Lys," insert --R_4 = Met, Val or Ala,--.

In claim 7 line 1, delete "one of the above claims," and insert therefore --claim 1,--.

In claim 8 line 1, delete "one of the above claims," and insert therefore --claim 1,--.

In claim 9 line 1, delete "one of the above claims," and insert therefore --claim 1,--.

In claim 10 line 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 11 line 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 12 line 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 13 lines 1 and 2, delete "one of claims 1 through 9," and insert therefore --claim 1,--.

In claim 14 lines 1 and 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 15 line 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 16 line 2, delete "claims 1 through 9" and insert therefore --claim 1--.

In claim 18 line 1, delete "claims 1 through 9" and insert therefore --claim 1--.

REMARKS

The claims have been amended to eliminate multiple dependency. Page 7 line 2 and claim 6 have been amended to add the definition of R_4. R_4 was previously defined at page 2 line 22 and claim 2 line 8, thus no new matter has been added. The drawings were substituted to conform with what happened during the international phase.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§1.16 and 1.17 which may be required during the entire pendency of this application, or to credit any overpayment, to Deposit Account No. 16-0820, Order No. 32409.

If any fees are required by this communication, please

charge such fees to our Deposit Account No. 16-0820, Order No. 32283.

Respectfully submitted,
PEARNE, GORDON, McCOY & GRANGER LLP

By: John P. Murtaugh
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Date: March 16, 2000

Figure 1

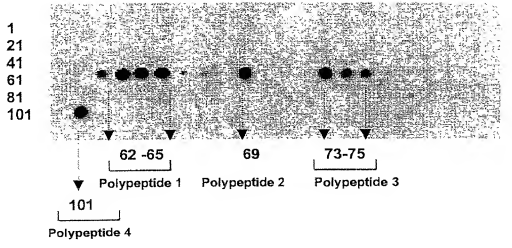


Figure 2

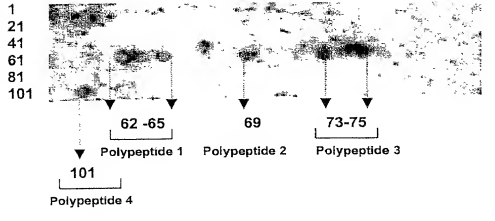
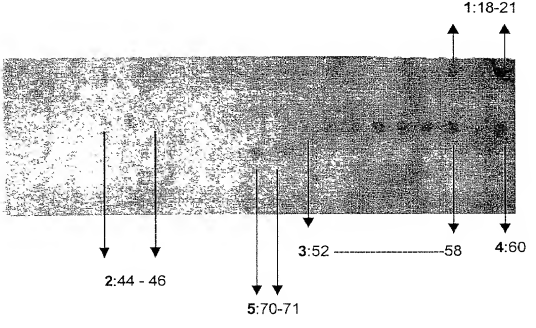


Figure 3



(6)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Markus Moser, Bruno Oesch, and Carsten Korth

Title: SYNTHETIC POLYPEPTIDE FOR DIAGNOSING AND TREATING PRION-RELATED DISEASES

Docket No. 32409

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS

I hereby declare that I am an officer of the concern identified below and am empowered to act on its behalf.

The concern qualifies as a small business concern as defined in 37 C.F.R. § 1.9 for purposes of paying reduced fees, in that the number of employees thereof, including those of its affiliates, does not exceed 500 persons.

The exclusive rights to the invention described and claimed in the above-identified application have been conveyed to and remain with the concern, or if the rights are not exclusive, all other rights belong to small entities as defined in 37 C.F.R. § 1.9.

I acknowledge the duty to file, in this application, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

PRIONICS AG

By: B. Oesch

Name: Dr. Bruno Oesch

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Date: 23 Feb 2000

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3/PRTS
514 Rec'd PCT/PTO 09/508828 16 MAR 2000

SYNTHETIC POLYPEPTIDE FOR DIAGNOSING AND TREATING PRION-RELATED DISEASES

The present invention relates to synthetic polypeptides and applies in particular to the diagnosis, prevention and therapy of several communicable, degenerative, neurological illnesses. Such illnesses are generically called spongiform encephalopathies, also prion illnesses. They occur in different mammals, for instance in the form of scrapie in sheep, BSE in cows and kuru or Jakob-Creutzfeld disease in humans.

The only molecule associated with the infecting agent found so far is a disease-specific prion protein (PrP^{Sc}) which is an anomalous isoform of a normal host protein (PrP^{C}) of unknown function. Both isoforms PrP^{Sc} and PrP^{C} coincide with respect to molecular weight and amino-acid sequence. They differ in the spatial folding and by their properties. Illustratively whereas PrP^{C} predominantly comprises α -helical secondary structures, and is soluble and protease-digestible, PrP^{Sc} comprises foremost β-sheet structures, and is insoluble and can be degraded only partly by proteases. Many indices, in particular the absence of other molecules except PrP^{Sc} , in the prion, and foremost the absence of nucleic acids, indicate that PrP^{C} assumes a significant (if not the main) role in initiating the above diseases. It is assumed that PrP^{C} proteins are able to convert normal PrP^{C} proteins into the disease-specific folding , thus explaining the infectiousness of PrP^{Sc} proteins.

Accordingly it appears very promising to develop therapies and diagnoses based on PrP^{Sc} as the central disease molecule.

Accordingly the objective of the invention is to create synthetic polypeptides offering the immunogenic properties, or in general the binding properties of PrP^{Sc} though free of its infectiousness.

This problem is solved by synthetic polypeptides defined in claim 1.

These are polypeptides containing one or more defined PrP sequences, where PrP denotes the prion protein generally independently of its conformation, these sequences being recognized by PrP^{Sc}-binding substances for instance in the mapping experiments which are described further below. There are a large number of different specifically PrP^{Sc}-binding substances. Examples are cited further below.

In summary the synthetic polypeptides of the invention therefore include at least one sequence which, in the native PrP^{Sc} is affixed to its surface where, alone or in combination with further sequences applicable within the scope of the invention, it shall form a binding site. At least one of the two β -sheet structures, or both, present in the structural model of the recombinant PrP, participate(s) in the formation of said PrP^{Sc}-specific surface structures. It is assumed that these structures act as a nucleation site in the PrP^{Sc} in the surface formation.

Synthetic polypeptides simulating binding sites present in the native PrP^{Sc} may be significant both in the therapy or diagnosis as well as regards prevention and other applications.

The invention in particular includes synthetic polypeptides comprising one or several of the following sequence segments stated in claim 2:

(a) Gly-R_1-Asp-R_2-Glu-Asp-Arg-(Tyr-Tyr)

(b) (Gln)-(Val)-Tyr-Tyr-R_3 -Pro-R_4-Asp-R_5 -Tyr-R_6-(Asn-Gln)

(c) Cys-R_7 -Thr-Gln-Tyr-R_8 -R_9-Glu-Ser-R_{10}-Ala-(R_{11} - Tyr)

(d) (Tyr-Arg)-Glu-Asn-Met-R_{12}-Arg-Tyr-Pro-Asn-(Gln-Val-Tyr)

where R_1= Asn or Ser, R_2= Trp or Tyr, R_3 = Arg or Lys, R_4 = Met, Val or Ala, R_5 = Gln, Glu or Arg, R_6 = Ser or Asn, R_7 = Val, Thr or Ile, R_8 = Gln or Glu, R_9 = Lys, Arg or Gln, R_{10} = Gln

or Glu, R_{11} = Tyr, Ser or Ala and R_{12} = His, Tyr or Asn, and where the amino acids in parentheses are not mandatorily present.

According to claim 3, further synthetic polypeptides used within the invention may contain one or more of the following sequences:

(e) Gly-Trp-Gly-Gln-Pro-His-Gly-Gly-Gly-Trp-Gly-Gln-Pro-His-Gly

(f) Lys-Pro-R_{14}-Lys-Pro-Lys-Thr-R_{14}-R_{15}-Lys-His-R_{16}-Ala-Gly

(g) Tyr-R_{16}-Leu-Gly-Ser

(h) Ser-Ala-Met-Ser-Arg-Pro-R_{17}-R_{17}-His-Phe-Gly-R_{14}-Asp

(i) Asn-Met-R_{18}-Arg-Tyr-(Pro-R_{14})-(Gln-Val-Tyr-Tyr-R_{19})

where R_{14} = Asn or Ser, R_{15} = Met, Leu or Phe, R_{16}= Met or Val, R_{17} = Ile, Leu or Met, R_{18} = His, Tyr or Asn and R_{19} = Lys or Arg and where the amino acids or sequence zones in parentheses are not mandatorily present.

The sequences of claims 2 and 3 were found in so-called mapping experiments on an immobilized peptide bank. As regards the peptide bank used (available from Jerini Biotools, Berlin), 104 peptides with 13 amino acids each are affixed by their C-terminal ends to a cellulose membrane. They cover the full sequence of PrP (hereafter PrP denotes generally the prion protein's basic amino-acid sequence regardless of conformation) and are configured in such a way as to be shifted each time by two amino acids, that is, each time 11 amino acids overlap between two adjacent peptide banks. In the course of several mapping experiments, peptide banks were loaded with different substances binding PrP^{Sc}, and the binding of these substances to special sequence zones was made visible using for instance a chemiluminescence kit (ECL, Amersham, USA).

In order to determine the sequences claimed in claim 2, a PrP^{Sc} -specific antigen denoted by 15B3 and (as found in our own pretesting, an also PrP^{Sc} -specific) recombinant bovine PrP (rbPrP) with the sequence shown in Fig. 4 were used as the PrP^{Sc} binding substances. To prepare rbPrP, illustratively a cell line (for instance E. coli) may be cultured with a vector expressing rbPrP in a suitable medium (for instance Luria broth) and then the prion protein may be isolated after being lysed from the cell inclusion bodies by further conventional purification procedures (see Homemann et al, FEBS Letters 97, 413 (2; 277-281)).

15B3 is a monoclonal PrP^{Sc} antibody recently discovered by the inventors. Hybridoma cells producing the said (PrP^{Sc} - specific) antibodies 15B3 were filed on 13 February 1997 as DSM ACC2298 at the German Collection of Microorganisms and Cell Cultures GmbH in Brunswick.

In both cases, the two differently binding substances, that is, the antibody 15B3 and the recombinant rbPrP, recognized as the same the sequences a-d of claim 1, as reproduced for 15B3 in Fig. 1 and for rbPrP in Fig. 2.

The numerals shown in Figs. 1 and 2 denote the different bank peptide sequences bound by the monoclonal antibody 15B3 and rbPrP. The sequences of the invention each correspond the zones common to the particular spatially adjacent binding peptides. As already mentioned, Fig. 2 shows the result of a mapping experiment of which the conditions correspond to the experiment represented in Fig. 1. In this instance the antibody 15B3 was merely replaced with recombinant bovine rbPrP. Because technical difficulties preclude better reproduction of the binding sites of the recombinant rbPrP, they are emphasized by marks. These are binding sites coinciding with those of Fig. 1.

The sequences stated in claim 3 also were determined by mapping experiments. However in this instance the recognizing substance is not an antibody or rbPrP, but instead it is the Congo Red dye of which the specific binding relating to PrP^{Sc} has already been known for some time (Prusiner et al, Cell 35, 349-358, 1983). Fig. 3 shows the corresponding peptide bank with the dyed binding zones from which, as stated above, the sequences e-i were determined.

It is clear from Figs. 1-3 that the sequences a-d and e-i are not linearly related PrP sequences. As regards a 3-D model of a C-terminal fragment of recombinant mouse PrP, it was found that two of the sequences a-d stated in claim 2 are spatially close to each other. It may be assumed with high probability that when the conformation is altered, the other two sequences also will assume another position in such a way that probably all four sequences a-d shall be configured near one another in the PrP^{Sc} and are highly likely to form a conformational epitope.

Accordingly the claimed sequences represent sequence zones recognized individually in a peptide bank for instance by a PrP^{Sc} -specific antibody and which moreover very probably constitute, individually or severally, a surface binding site, for instance an epitope, in the native PrP^{Sc} -protein. The expression "epitope" denotes the specific antigen site on the surface of the PrP^{Sc} -protein which illustratively can be bound by the idiotype of 15B3.

As a result the invention prepares synthetic polypeptides which at a minimum contain one of the said PrP^{Sc} -binding substances in the sequences recognized in the peptide bank, as well as any additional ones.

Synthetic polypeptides comprising a PrP^{Sc} antigen zone already have been disclosed in the patent document WO 93/11153. The sequences stated therein represent comparatively

substantial segments of the PrP sequence. The precise boundary of a sequence for instance forming an epitope or participating in it, is lacking, and this feature hampers or makes impossible in particular the spatial buildup of minimal synthetic polypeptides having for instance the immunogen effect of PrP^{Sc}.

As discussed above, at a minimum, the synthetic peptides may be composed merely of one of the sequences claim 2 or one of the cited ones. However they may also be bound to further, suitable sequences which hereafter are called conformation sequences.

Theoretically the sequences for instance might be connected to each other illustratively by means of said conformation sequences and possibly by further sequences in such manner as to stimulate the presumed spatial configuration in the PrP^{Sc} protein. Ideally a protein (epitope) would be attained in this manner which would contain several neighboring binding sites as is the case for the PrP^{Sc}.

However, in one implementation of the invention, only one of the claimed sequences (sequence b) shall be so connected to a conformation sequence that a synthetic polypeptide is made that offers adequate binding for instance regarding 15B3, as confirmed by the inventors' tests. A polypeptide of such a configuration may include one of the two following sequences:

(j) (X)-(Gly)-Ala-Val-Val-Gly-Gly-Leu-Gly-Gly-Tyr-(R_{13})-Z-Tyr-Tyr-R_3-Pro-R_4-Asp-R_5-Tyr-R_6- (Asn-Gln)-(Y)

(k) (X)-Tyr-Tyr-R_3 -Pro-R_4-Asp-R_5 -Tyr-R_6 -(Asn-Gln)-Z-(Gly)-Ala-Val-Val-Gly-Gly-Leu-Gly-Gly-Tyr-(R_{13})-(Y)

where X and Y are arbitrary amino-acid sequences, Z is a conventional spacer, for instance Gly-Gly, R_3 = Arg or Lys, R_5 = Gln, Glu or Arg, R_6 = Ser or Asn and R_{13} = Met or Val and where the sequence zones in parentheses are not mandatorily present.

The j-sequence contains in its C-terminal zone the sequence b which is connected for instance by means of the spacer Gly-Gly to the adjoining N-terminal conformation sequence. The order is exactly the opposite in the k sequence. Other appropriate spacers in general are those assuring adequate flexibility between the connected peptide zones and exerting no influence on conformation.

Both preferentially used synthetic peptides were designed on the findings that β sheet structures occur in increased manner in PrP^{Sc}, practically always a conformation sequence inducing a β sheet structure being present up or down the sequence. The synthetic polypeptides of claim 6 therefore were provided as claimed in claim 6 with suitable conformation sequences in order to configure the epitope sequence in a β sheet structure specific for PrP^{Sc}.

In well known manner, depending on their size, polarity or charge, amino acids may be assigned into different groups. The amino acids within one group are said to be mutually homologous and there are five groups:

(1) small aliphatic, non-polar or only slightly polar acids: alanine, serine, threonine, and, within limits, glycine, proline

(2) polar, negatively charged acids and their amides: aspergillic acid, asparagine, glutamic acid and glutamine

(3) polar, positively charged acids: histidine, arginine, lysine

(4) large aliphatic, non-polar acids: methionine, leucine, isoleucine, valine, cysteine,

(5) large aromatic acids: phenylalanine, tyrosine, tryptophane.

In many cases it is possible to replace amino acids contained in peptide sequences by corresponding acids from the same group without thereby entailing a change in sequence properties. Therefore the invention also includes those sequences that do not correspond to the explicitly stated formulas but wherein one or more amino acids were replaced by a homologous acid.

Another justifiable assumption is that independently of their direction of formation, amino-acid sequences under given circumstances may offer similar binding properties, in particular antibody binding properties. In such a case they are called "retro-aminoacid sequences" which denote coinciding sequences formed in a C terminal or N terminal direction (for instance [N-terminal]- Glu-Ala-Val-Leu-[C-terminal], [N-terminal]-Leu-Val-Ala-Glu - [C-terminal]). If the amino acids being used are present in D chiral form opposite the L form of animals, then the epitope zones will be mirror symmetrical and shall also be recognized by a few antibodies, the isotypes of these antibodies differing in these properties. In such cases the terminology is "inverso-aminoacid-sequences. When both inverso and retro amino acids are used, there will be for instance coinciding epitope zones which can be unrestrictedly bound of the antibody which is specific to the original sequence. The advantage offered for instance by such retro-inverso sequences of amino acids is that the D amino acids are degraded more slowly by the organism because being recognized more poorly by the degrading enzymes. The same effect can also be achieved by substituting non-natural amino acids. Therefore the peptides of the invention also may be in retro- and/or inverso form and moreover they may contain non-natural amino acids, that is not produced by organisms. Non-

natural amino acids may be prepared by synthesizing for instance additional side chains or reactive groups in a manner to offer specific properties and matched to specific applications.

As already discussed above, the synthetic polypeptides of the invention may be used in particular in the treatment, prevention or also diagnosis of prion diseases.

A particular application is to use the synthetic polypeptides of the invention as vaccines. Illustratively an appropriate quantity of peptide is dissolved using Freund's complete adjuvant injecting it sub-cutaneously or intra-muscularly. At intervals of several weeks, again an immunogenic quantity of peptide is dissolved in Freund's incomplete adjuvant and is injected (boost). The objective of this vaccination is to induce an immune response, including endogenous production of antibodies able to specifically recognize PrP^{Sc} and neutralize or characterize it, whereby the body's own defense mechanism shall be able to forestall disease or slow it or stop it.

Another application consists in using the synthetic polypeptides in diagnosis and therapy. In the light of the prevailing conversion theory, it is assumed the PrP^{Sc} and/or PrP^{C} also bind to each other. This supposition is supported by further mapping experiments by the inventors showing (Fig. 2) that recombinant bovine rbPrp binds to sequence zones similar to those that the above antibody 15B3 binds with.

The said binding properties may be put to use for instance in therapy. Conceivably the polypeptides may be cerebrally applied to an ill patient and there they shall be available as a binding partner to the infectious PrP^{Sc}. In this manner the conversion rate might be sharply reduced and the progress of the disease slowed down. As regards diagnosis, any PrP^{Sc} contained in sample material might be specifically bound by means of the polypeptides of the invention and then be detected in appropriate manner.

The synthetic polypeptides of the invention are not restricted to the above cited sequences. Illustratively peptides in derivative form also are applicable. Illustratively such peptides might be bound to a carrier, or immunogen, such as diphteriatoxin or BSA to enhance the immune response. Another way to make derivatives is by linking to markers, for instance using biotine or peroxidase or enzymes or nucleic acids. Lastly, signal sequences might be used to facilitate the passage of the peptides into desired compartments. This latter application relates in particular to the blood/brain barrier which might be easier to cross when using signal sequences binding the transferrin receptor.

As already stated above, the synthetic polypeptides of the invention are applicable to the therapy, diagnosis and prophylaxis of prion diseases. In conjunction with all said applicabilities, it is essential that the polypeptides of the invention be administered to the patients per se or in combination with further substances, and, as already mentioned, derivatives may be used to enhance the directionality into specific compartments.

The polypeptides may be manufactured in arbitrary manner: either directly through conventional peptide-syntheses or also indirectly through RNA or DNA synthesis and then by conventional molecular-biological techniques. Accordingly another feature of the invention relates to a DNA molecule which is able to code one of the polypeptides of the invention. Preferably such a DNA molecule (where called for also in a longer sequence) is made available in an appropriate expression vector. The routines involved are conventional.

The invention furthermore relates to a kit to diagnose PrP^{Sc} or antigens against PrP^{Sc} and containing at least one of the polypeptides of the invention. This feature avails itself of the fact that the polypeptides can specifically bind with the PrP^{Sc} and with the antibodies pointing at it.

As already mentioned, one of the substances used to ascertain the polypeptide sequences may be recombinant bovine rbPrP. Surprisingly it was found that recombinant rbPrP is able to specifically bind to PrP^{Sc} and to recognize, at the corresponding peptide bank, the same sequences as the antibodies 15B3 (see Fig. 2).

Another feature of the invention therefore relates to the use of recombinant rbPrp corresponding to the sequence of Fig. 4. It was found that PrP^{Sc} specific antibodies are produced when administering recombinant bovine rbPrp of the indicated sequence. This effect can be exploited in particular with respect to prophylaxis or therapy in that recombinant rbPrP prepared as a vaccine is administered to a patient and thereby a corresponding immune response shall be triggered.

Obviously the implementation is not restricted to using bovine rbPrP per Fig. 4. Recombinant PrP sequences with species-specific deviations from the rbPrP sequence shown in Fig. 4 may be used just as well.

Lastly the invention relates also to a method for manufacturing PrP^{Sc}-specific antibodies. For purposes of immunization, at least one of polypeptides of the invention is administered in a dose sufficient for immunization to non-human mammals and the antibody thereby formed is then isolated in conventional manner.

Lastly the peptides of the invention also are suitable for the so-called pharmaceutical or chemical libraries whereby new active ingredients are tested or determined which shall specifically bind PrP^{Sc}.

SEQUENCE PROTOCOL

(1) General data

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(ii) Title of Invention: Synthetic polypeptide for diagnosing and treating prion-related diseases.

(iii) Number of sequences : 1

(iv) Computer readable version:
(a) data medium: floppy disk
(b) computer: IBM PC compatible
(c) operating system: PC-DOS/MS-DOS
(d) software: PatentIn Release # 1.0, version #1.30 (EPA)

(vi) Data of first application:
(a) Application # DE 19741607.1
(b) Date of application: 20 September 1997

(2) DATA RELATING TO SEQ ID # 1:

(i) SEQUENCE CHARACTERISTICS
(a) Length: 219 amino acids
(b) Species: Amino acid
(c) Form of strand: single strand
(d) Topology: Linear

(ii) MOLECULE SPECIES: protein

(iii) hypothetical: yes

(iv) antisense: no

(vi) PROVENANCE:
(a) organism: bos taurus

(vii) GENOME POSITION:
(c) units: 219

RSNZ 240213

(xi) SEQUENCE DESCRIPTION: SEQ ID #: 1:

```
Met Lys Lys Arg Pro Lys Pro Gly Gly Gly Trp Asn Thr Gly Gly Ser
1               5                   10                  15
Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg Tyr Pro Pro Gln
            20                  25                  30
Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro
        35                  40                  45
His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro
    50                  55                  60
His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln
65                  70                  75                  80
Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn
                85                  90                  95
Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly
            100                 105                 110
Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His
        115                 120                 125
Phe Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg
    130                 135                 140
Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln
145                 150                 155                 160
Asn Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Glu His Thr
                165                 170                 175
Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys
            180                 185                 190
Met Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg
        195                 200                 205
Glu Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser
    210                 215
```

CLAIMS

1. A synthetic polypeptide containing one or several defined sequences of PrP or sequences derived therefrom, said sequences being recognized by PrP^{Sc} - binding substances.

2. Synthetic polypeptide as claimed in claim 1, wherein the sequence corresponds to one of the following formulas, containing at least one of the said sequences or a combination of several sequences:

(a) Gly-R_1-Asp-R_2-Glu-Asp-Arg-(Tyr-Tyr)

(b) (Gln)-(Val)-Tyr-Tyr-R_3 -Pro-R_4-Asp-R_5 -Tyr-R_6-(Asn-Gln)

(c) Cys-R_7 -Thr-Gln-Tyr-R_8 -R_9-Glu-Ser-R_{10}-Ala-(R_{11}-Tyr)

(d) (Tyr-Arg)-Glu-Asn-Met-R_{12}-Arg-Tyr-Pro-Asn-(Gln-Val-Tyr)

where R_1= Asn or Ser, R_2= Trp or Tyr, R_3 = Arg or Lys, R_4 = Met, Val or Ala, R_5 = Gln, Glu or Arg, R_6 = Ser or Asn, R_7 = Val, Thr or Ile, R_8 = Gln or Glu, R_9 = Lys, Arg or Gln, R_{10} = Gln or Glu, R_{11} = Tyr, Ser or Ala and R_{12} = His, Tyr or Asn, and where the amino acids in parentheses are not mandatorily present.

3. Synthetic polypeptide as claimed in claim 1, wherein the sequence corresponds to one of the following formulas, containing at least one of the said sequences or a combination of several sequences:

(e) Gly-Trp-Gly-Gln-Pro-His-Gly-Gly-Gly-Trp-Gly-Gln-Pro-His-Gly

(f) Lys-Pro-R_{14}-Lys-Pro-Lys-Thr-R_{14}-R_{15}-Lys-His-R_{16}-Ala-Gly

(g) Tyr-R_{16}-Leu-Gly-Ser

(h) Ser-Ala-Met-Ser-Arg-Pro-R_{17}-R_{17}-His-Phe-Gly-R_{14}-Asp

(i) Asn-Met-R_{18}-Arg-Tyr-(Pro-R_{14})-(Gln-Val-Tyr-Tyr-R_{19})

where R_{14} = Asn or Ser, R_{15} = Met, Leu or Phe, R_{16}= Met or Val, R_{17} = Ile, Leu or Met, R_{18} = His, Tyr or Asn and R_{19} = Lys or Arg and where the amino acids or sequence zones in parentheses are not mandatorily present.

4. Synthetic polypeptide as claimed in one of claims 1 through 3, characterized in that the sequence is coupled with a "conformation" sequence, where applicable by means of a conventional spacer sequence, said conformation sequence inducing the formation of a defined conformation of the synthetic polypeptide.

5. Synthetic polypeptide as claimed in one of claims 1 through 4, characterized in that the "conformation" sequence induces the formation of a β strand.

6. Synthetic polypeptide as claimed in claim 2, 4 and 5, corresponding to one of the following formulas:

(e) (X)-(Gly)-Ala-Val-Val-Gly-Gly-Leu-Gly-Gly-Tyr-(R_{13})-Z-Tyr-Tyr-R_3 -Pro-R_4-Asp-R_5 -Tyr-R_6 -(Asn-Gln)-(Y)

(f) (X)-Tyr-Tyr-R_3 -Pro-R_4-Asp-R_5 -Tyr-R_6 -(Asn-Gln)-Z-(Gly)-Ala-Val-Val-Gly-Gly-Leu-Gly-Gly-Tyr-(R_{13})-(Y)

where X and Y are arbitrary amino-acid sequences, Z is a conventional spacer such as Gly-Gly, R_3 = Arg or Lys, R_5 = Gln, Glu or Arg, R_6 = Ser or Asn and R_{13} = Met or Val, and where the sequence zones in parentheses need not necessarily be present.

7. Synthetic polypeptide as claimed in one of the above claims, characterized in that it is present in the retro form at least in one partial sequence.

8. Synthetic polypeptide as claimed in one of the above claims, characterized in that at least one of the amino acids it contains is present in the D form.

9. Synthetic polypeptide as claimed in one of the above claims, characterized in that it is present in derivative form.

10. A pharmaceutical preparation for the therapy of prion diseases, characterized in that it contains at least one of the synthetic polypeptides stated in claims 1 through 9 or at least one PrP^{Sc}-binding substance recognizing the defined sequences, and contains it in a dose adequate for therapy or prevention.

11. Diagnostic means for prion diseases, characterized in that it contains at least one of the synthetic polypeptides stated in claims 1 through 9 or at least one PrP^{Sc}-binding substance recognizing the defined sequences in a dose sufficient for the particular detection.

12. Diagnostic means for prion diseases, characterized in that it contains at least one of the synthetic polypeptides stated in claims 1 through 9 or at least one PrP^{Sc}-binding substance recognizing the defined sequences in a dose sufficient for immunization.

13. A pharmaceutical preparation, a diagnostic means or vaccine as claimed in one of claims 1 through 9, characterized in that the PrP^{Sc} - binding substance it contains is a recombinantly produced rbPrP of the formula of Fig. 4 or in the form of genus-specific deviations thereof.

14. A DNA molecule coding at least one of the synthetic polypeptides of claims 1 through 9.

15. A kit to detect PrP^{Sc} or antibodies recognizing it, characterized in that it contains at least one synthetic polypeptide as claimed in claims 1 through 9.

16. A method for preparing PrP^{Sc}-specific antibodies characterized in that non-human mammals are immunized with at least one polypeptide as claimed in claims 1 through 9 and in that the antibody or antibodies formed as a reaction are conventionally isolated from the mammal following a time interval sufficient for immunization.

17. A method for detecting PrP^{Sc}-specific surface sequence zones, characterized in that a PrP^{C}-specific peptide bank is incubated with PrP^{Sc}-binding substances and in that the binding zones of the peptide bank are made visible using usual visualization techniques and in that the sequence zones are determined therefrom.

18. Application of the polypeptides claimed in claims 1 through 9 to a pharmaceutical or chemical library to detect PrP^{Sc}-specific active ingredients.

ABSTRACT

A synthetic polypeptide containing one or more defined sequences of PrP or derivative sequences that are recognized by PrP^{Sc}-binding substances.

Figure 1

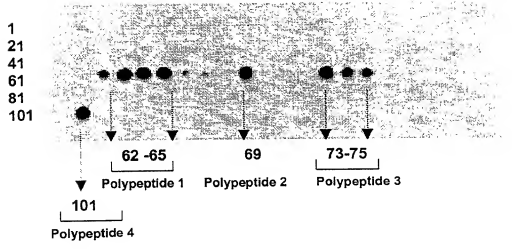


Figure 2

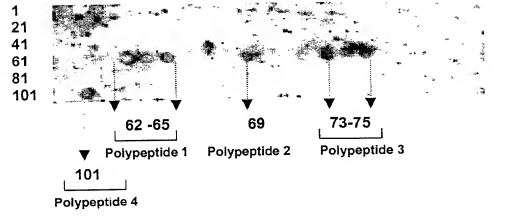
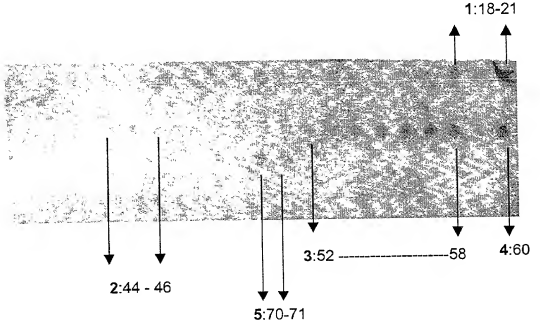


Figure 3



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DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION

[ ] Submitted with Initial Filing

[X] Submitted after Initial Filing (Surcharge (37 CFR 1.16(e)) required)

Attorney Docket No.: 32409

First Named Inventor: Markus Moser

Application Number: 09/508,828

Filing Date: March 16, 2000

Group Art Unit:

Examiner Name:

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYNTHETIC POLYPEPTIDE FOR DIAGNOSING AND TREATING PRION-RELATED DISEASES

PATENTANWÄLTE SCHAEFER & EMMEL
ENG. 07 JUL 2000
REC.
TECH: U T

the specification of which (check only one item below)

[ ] is attached hereto,

OR

[X] was filed on (MM/DD/YYYY) September 17, 1998 as United States Application Number or PCT International Application Number PCT/EP98/05924 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d), or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Country	Prior Foreign Application Number(s)	Foreign Filing Date (MM/DD/YYYY)	Priority Claimed?
DE	197 41 607.1	September 20, 1997	Yes

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